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PLAN B THESIS

STATISTICAL MODELLING METHODOLOGY FOR THE DETERMINATION OF HABITAT SUITABILITY AND HABITAT PREFERENCES OF THE ENDANGERED FOUNTAIN DARTER

by

Michael E. Chulick

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ABSTRACT

The San Marcos and Comal Rivers, located in south Texas, support populations of five Federally listed or endangered species. Both rivers are fed by spring runs which are supplied by the Edwards aquifer which is currently in an overdraft condition. A major reduction or elimination of spring flows is considered a severe threat to the survival of these species. In order to preserve these species, the United States Fish and Wildlife Service (USF&WS) initiated a 5-year cooperative agreement with Utah State University to assess and quantify the instream flows necessary to protect these species.

The research presented in this paper concentrates on the habitat needs and habitat utilization patterns of the fountain darter (*Etheostoma fonticola*) in the Comal River with the goals of generating a habitat occupancy equation, a population density equation, and developing a statistical methodology for handling similar situations. Field data collected on the Comal over a one year period contained information on the physical, chemical, and vegetative environment as well as information on fish species composition and numbers. This data was systematically reduced, normalized, and then analyzed by various statistical techniques including principle component analysis, discriminate functions analysis, multiple regression analysis, and analyses of variance and covariance.

An occupancy equation (presence/absence) was successfully developed with a 78% accuracy rate for the summer season and a 58% accuracy rate for the fall season. A statistically valid population density equation was only developed for the summer season and had a model R² of 0.28.

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INTRODUCTION

The San Marcos and Comal Rivers, located in south Texas (Figure 1), support populations of five Federally listed threatened or endangered species. One species is currently listed as endangered. These rivers provide unique habitat as a result of their common source, the Edwards Aquifer. This aquifer is currently in an overdraft state which jeopardizes the flow from the springs which feed these rivers and thus the entire aquatic ecosystem. One of the species, the fountain darter (Etheostoma fonticola) was reintroduced into the Comal River from the San Marcos after going locally extinct in the 1950's due to reduced spring flows. For this reason, as well as other pressures on these ecosystems, the U.S. Fish and Wildlife Service (USF&WS) initiated the San Marcos and Comal Springs and Associated Aquatic Ecosystems Recovery Plan (Aug 1994). One of the major actions required in this plan is to determine the instream flow needs of flow dependent aquatic resources of the Comal and San Marcos ecosystems. As part of this effort, the USF&WS initiated a 5-year cooperative agreement with Utah State University to assist in research on the development and application of multidisciplinary assessment methods for these river systems with the overall goal of quantifying instream flows necessary to protect these unique aquatic ecosystems. A comprehensive study plan (Hardy 1992) was developed in consultation with species experts, local, state, and federal management agencies, and other interested parties within the environmental community which has subsequently been implemented. The scope of work outlined in the study plan focused on research to characterize the physical, chemical, and biological environment within the Comal and San Marcos River

Ecosystems and subsequent development of assessment tools appropriate for evaluation of impacts of alternative flow levels on target species of concern.

This thesis is intended to support elements of this research effort within the Comal River Ecosystem relating specifically to the habitat needs and habitat use patterns of the fountain darter (Etheostoma fonticola). Developing a mathematical representation of suitable darter habitat which can be used in conjunction with instream flow assessment methods also under development will enable the USF&WS to evaluate discharge levels for the springs which optimize the opportunity for species survival and ecosystem protection.

Life History Requirements of the Fountain Darter (Etheostoma fonticola)

The amount of knowledge concerning the habitat needs and preferences of the fountain darter is somewhat limited. The USF&WS recovery plan contains a summation of what is known about the species, with the pertinent elements that relate to this thesis summarized below. The fountain darter is a small species of darter occurring exclusively in the San Marcos and Comal Rivers (Figure 1). The species is considered to be the most advanced or specialized of the darters based on an analysis by Bailey and Gosline (1955) and Collette (1962) which showed some of the species traits to be highly influenced by environmental conditions. The USF&WS recovery plan states the following concerning fountain darter habitat requirements:

- 1) undisturbed stream floor habitats (including runs, riffles, and pools)
- 2) a mix of submergent plants and mats of filamentous algae, in part for cover

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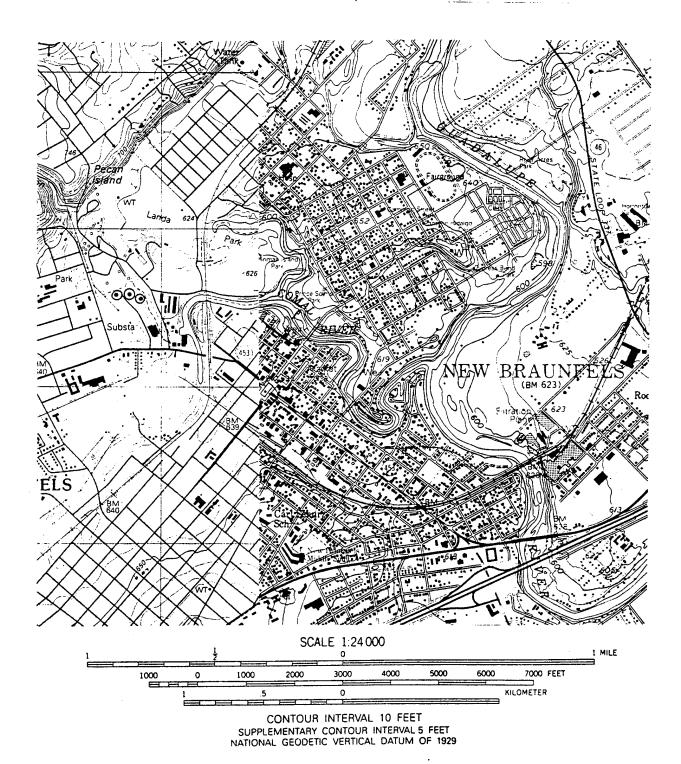


Figure 1. Comal River Study Location

- 3) clear and clean water
- 4) food supply of living organisms
- 5) most importantly, adequate springflows.

Based on field observations, the fountain darter prefers the stream bottom, constant water temperature, and mats of vegetation, most notably, filamentous green algae and the bryophyte <u>Riccia</u>.

Sources of Data

To improve the knowledge of what constitutes suitable darter habitat, members of the USF&WS, the Texas Department of Parks and Wildlife and Utah State University collected extensive field data within the Comal Rivers during four sampling periods (July and November 1993, January and April 94). The river was first photographed aerially using multi-spectral imaging and divided into strata (reaches) based on similarity of channel conditions. Each strata was then divided into uniquely numbered, 10 m² cells. Figure 2 shows a typical aerial photograph of the Comal; specifically stratas 8, 5, and part of strata 4 with the grid system imposed. A minimum of ten percent of these cells from each strata were randomly selected for sampling during each period. Sampling was accomplished using a 1m long by 2 m wide by 2 m tall rectangular drop net structure placed into each sampled grid location. Cells with a depth exceeding 2 m were not sampled. The data gathered includes information on the physical and chemical conditions of each grid, amount and type of vegetation, population and species composition of fish, and species composition of invertebrates

present. Invertebrate data was not included in this analysis. Specific information on the methods used to gather this data is contained in the <u>Habitat and Flow</u>

Requirements Study for the Comal Ecosystem (Hardy, 1992) which is provided in Appendix 1. The field data collected can be roughly grouped into four categories or attribute types: physical, chemical, vegetative, and fish. The information gathered on each cell is shown in Table 1.

Table 1. Field Data Collected

PHYSICAL	CHEMICAL	VEGETATIVE	FISH
Depth	Water Temperature	Species Composition	Population Numbers
Mean Column Velocity	Turbidity	% Aerial Coverage	Species Composition
Bottom Velocity @ 15 cm	Specific Conductivity	Height Above Bottom	Darter Lengths
Substrate	рH		
	Dissolved Oxygen		

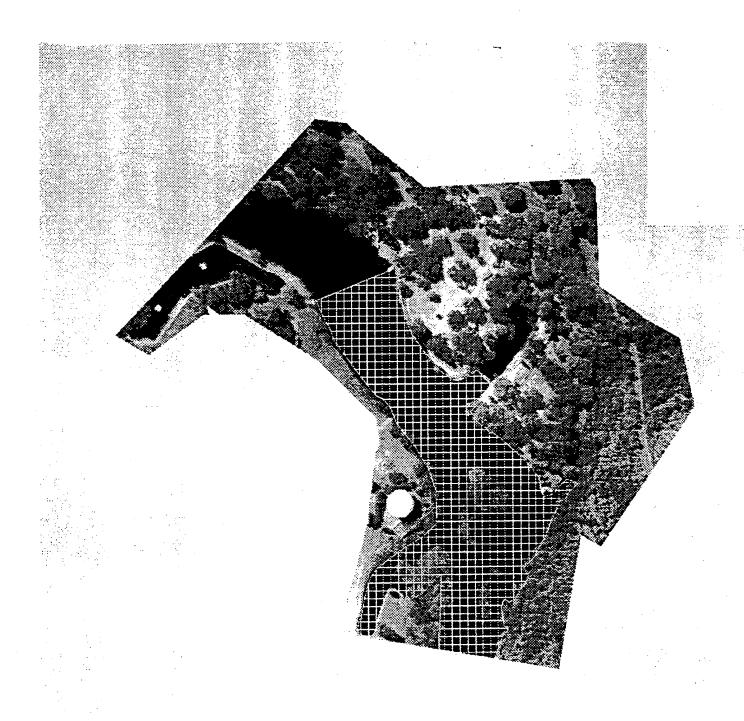


Figure 2. Aerial Photograph of the Comal with Grid Overlay

PROJECT GOALS

The goals of this project are threefold:

- Develop a logical statistical modeling methodology for the evaluation of this data set and for future applications.
- 2. Develop a habitat occupancy equation for the Comal River darter.
- 3. Develop a population density equation.

Accomplishment of these goals will provide the USF&WS with the information it requires to integrate biological criteria with hydraulic and other physical habitat modeling efforts to evaluate various discharge levels which optimize fountain darter habitat within the Comal River ecosystem as well as a methodology for evaluating similar requirements within the San Marcos River Ecosystem.

METHODS OF ANALYSIS

Some of the most common approaches to predicting habitat suitability include the calculation of a Habitat Suitability Index (HSI) and the closely allied Habitat Capability (HC) models (Morrison 1992). The calculated values from these procedures represent the species response to the combination of key variables selected by the investigator. These methods assume that the variables chosen adequately represent a species response to the environment but does not typically provide information on population size. These procedures do not lend themselves to application in this study

as little is known concerning the habitat requirements of the darter and therefore selection of the key parameters required for the procedures would be guesswork.

More powerful statistical techniques commonly used by biologists include principle component analysis (PCA), multivariate regressions, and discriminate functions analysis (Morrison 1992). In particular, multiple regression is the most widely used and misused technique employed by biologists due to its predictive power (Pimentel 1979). Morrison (1992) states that the primary cause of misinterpretation and misuse of regression is multicollinearity. He further states that multicollinearity is "especially evident in wildlife-habitat analysis" due to the fact that multiple regression is used as an exploratory tool to identify important variables from a larger set of variables. Further errors are introduced by failure to conduct proper residual analysis.

As long as one accounts for the difficulties in using multiple regression techniques to assess biological data, they do provide an exceptional tool for examining complex data sets such as those in this study. The basic techniques employed in this study are discriminate function analysis, principle component analysis, and multiple variable regression. The framework for how these techniques were used to evaluate the field data is shown below in Figure 3.

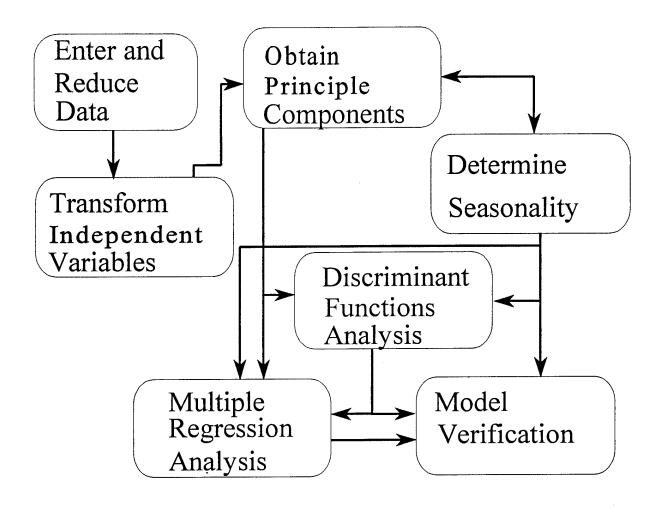


Figure 3. Data Analysis Framework

The raw data was entered into data base files then exported to Borland's Quattro Pro™ spreadsheet files for manipulation. Average velocities were calculated, darter densities tabulated, and any data from those reaches heavilly influenced by spring run imput removed. The data from the strata dominated by spring run flows were removed from the data set as they did not comprise habitat similar in nature to the main Comal River channel. Average velocities, when not collected in the field, were

calculated by taking the numerical average of the velocity measured in the field at 0.2 and 0.8 of the column depth. The absolute value of all velocity measures (measured and calculated) was then taken to remove the directional component. The variable Darter was also created and represents the total number of all darters caught in all the sampling passes conducted on a grid cell. The modified data sets were then exported to SAS Institute Inc.'s SAS™ for further data manipulation and statistical anlyses.

Due to the number of vegetative species (23) and the known importance of vegetation to darters, it was necessary to develop new variables that adequately represented the vegetative community observed in the field while reducing the total number of variables involved. The species of vegetation observed were divided into three broad structural classes. Since each grid contained information concerning the percent of vegetative cover, the dominant species, and the subdominant species of vegetation; the three vegetative community variables are also needed to adequately reflect the true information provided by the raw variables. To accomplish this, a 75%/25% split of total cover was used to assign percentages to dominant/subdominant vegetative cover. When no subdominant species was present, all cover was assigned to the dominant species. The total vegetative cover for a grid cell is composed of the sum of the three vegetative communities. The species included in each vegetative community are shown in Table 2.

Table 2. Plant Species Composition of Vegetative Communities

	<u>, </u>	
COMMUNITY 1	COMMUNITY 2	COMMUNITY 3
Riccia fluitans	Vallisneria americana	Ludwigia repens
Cambomba caroliniana	Sagittaria platyphyla	Nuphar luteum
Utricularia gibba		Ceratopteris thalictroides
Amblystegium riparium		Sagittaria playtphyla
Rhyzoclonium		Hydrocotyle
unidentified moss		chara species
algae, various species		Potamogeton
		Justicia americana
		Eleocharis
		Rorippa n-aquaticum
		unidentified grass
		Colocasis esculenta
		Polygonum
		unidentified iris/flag

Finally, two categorical variables were assigned to each sampling location.

Present (1 or 0) indicates whether darters were present or absent in any sampled cell while Period (1=july,2=oct, 3=jan, 4=april) associates the site with a sampling season.

The content and structure of the analysis files used is shown in Appendix 2. The actual files are stored on the 3.5 inch diskette accompanying this document.

The first step in the analysis following data entry was to conduct transformations of the independent variables to achieve as nearly as possible a normal distribution for each. The requirement for multivariate normality of the independent variables is

paramount for the successful application of principle components analysis and discriminate functions analysis. SAS™ incorporates a number of numerical and graphical normality tests into its univariate procedure. The measures considered during testing of the variables during this study were Shapiro and Wilk's (1965) W-statistic and normal-probability plots. For the W-statistic, a value of unity indicates a perfectly normal distribution. The normal-probability plot graphically compares the observed quantiles for a given variable against the corresponding quantiles for a theoretical Gaussian distribution with the same mean and standard deviation.

Departures from the diagonal indicate deviations from the normal distribution. The values of the W-statistic for the raw data are presented in Table 3.

Table 3. Normality Test Results (W) for the Raw Data (All Seasons)

VARIABLE	W	VARIABLE	W
рН	0.969	Velocity-Bottom	0.526
Depth	0.969	Velocity-Average	0.605
Temperature	0.867	Veg Community 1	0.563
Dissolved Oxygen	0.975	Veg Community 2	0.351
Specific Conductivity	0.692	Veg Community 3	0.747
Darter Density	0.594		

Based on the W statistic, data transformations were necessary for all variables except ph, depth, and dissolved oxygen. The shape of the normal-probability curve indicated which direction to transform the data (positive or negative skew) as well as whether a transformation was even likely to work. Transformations were expected to work on all variables except the three vegetative communities. The plots for these variables

transformations. The multi-modal nature of these variables may be attributed to the manner in which they were derived. Each is a function of the field estimation of percent vegetative cover based on ocular estimates by the field crew. The estimates were much closer to categorical (0,25,50, 75, 100%) than continuous. However, it was necessary to treat them as continuous variables in order to use them in the analysis.

The results of the data transformations on univariate normality are presented in Table 4. The transformations were used on the entire data set first then on each individual sampling period to ensure that they remained valid. The mean value of W for the four periods as well as the associated standard deviation is also included.

Table 4. Normality Test Results on Transformed Data

VARIABLE	W Whole	W July	W Oct	W Jan	W April	W AVG	S.D. _a
log ₁₀ (avg velocity)	.959	.954	.965	.956	.954	.957	.004
log ₁₀ (darter + 1)	.850	.864	.787	.871	.860	.845	.034
temperature ³	.951	.953	.741	.941	.952	.897	.090
spec. cond ⁻³ _b	.884	.651	.756	.745	.882	.758	.082
log ₁₀ (bottom velocity)	.907	.890	.944	.906	.888	.907	.022
arcsin(sqrt vc1) _c	.627	.625	.593	.644	.690	.638	.035
arcsin(sqrt vc2) _c	.370	.375	.297	.488	.287	.362	.080
arcsin(sqrt vc3) _c	.785	.782	.782	.769	.805	.785	.013

a. S.D. - Standard Deviation

The selection of the arcsin of the square root of the vegetative communities seemed the most appropriate transformation as the data reflects percentages from a non-

b. spec. cond. - specific conductivity

c. vc - vegetative community

normal, possibly binomial, distribution. Zar (1984) states that this transformation yields a nearly normal distribution for percentages from binomial data. While this turned out not to be the case for this data set, it did yield marginal improvement in the value of W.

Once the independent variables were transformed, Principle Components

Analysis (PCA) was conducted on the whole data set as well as for each of the seasons. The components obtained in this analysis represent linear combinations of the transformed independent variables that account for the maximum variation in the data. Each vector component exhibits the characteristic of being orthogonal to the other vector components in the n-dimensional space being explained which removes concerns about correlation and multicollinearity.

The next step in the analysis was to determine if the data set could be treated as one data set or broken down according to sampling periods due to variations between seasons. An analysis of variance (ANOVA) was conducted on the principle components obtained for the whole data set (all seasons) as well as the transformed independent variables. The results of this analysis determined how the data was grouped for all subsequent analyses.

Following the ANOVA of seasons, two presence/absence models describing suitable darter habitat were developed for each season observed using discriminate functions analysis (DFA). The first model consists of the transformed independent variables while the second is comprised of principle components. The occupancy models were evaluated against the observed data as shown in Table 5.

Table 5. Occupancy Model Evaluation

	True Distribution of Fountain Darters				
Model Prediction	Species Present	Species Absent			
Present	Correct	Correct Type I Error			
Absent	Type II Error	Correct			

Morrison (1992) states that Type I errors may occur due to incorrect sampling methods, strategy, or timing; the species is inherently rare and does not occupy all suitable habitats; or the model overstated the value of a set of conditions or missed a detrimental condition. Type II errors occur due to species wandering, sampling design, or the model did not include a vital environmental parameter.

The basic approach to the problem of generating a population density equation was to use multiple regression anlysis on both the untransformed raw data and the principle components for each season with observed darter populations as the response variable. Two variations of the original data set were modeled. The control technique was a traditional multiple regression using the entire unmodified data set for each season. The experimental technique was a two-step hierarchical approach (Cavalli and Crowl, in review) which attempts to account for the assumption that there exists suitable habitat that is not occupied by darters and to include this unoccupied habitat in a regression model predicting population density. This technique, and the focus of this study, was to attempt a regression using all occupied habitats plus only those vacant habitats that were mathematically determined to be suitable for the fountain darter. This method employs the equations predicting presence/absence from the DFA analysis to predict which of the vacant habitats should have contained darters.

These sites are included with the occupied sites in the regression analysis. Sites which did not have darters observed and were also predicted not to have darters were excluded from the regression analysis. The modified data set will be referred to as the DFA-modified data for the period in question in all subsequent discussions. All regression efforts were analyzed for the effects of multicollinearity as well as normality of residuals to insure that the basic mathematical assumptions of the model employed were met.

The final step in the analysis was to validate the equations generated against any subsequent sampling periods that were considered statistically the same according to the ANOVA previously accomplished. The presence/absence equations were validated using a contingency table to generate chi-square values. If the chi-square value generated is less than the critical chi-square value, then the null hypothesis was not rejected and the populations were considered indistinguishable; the equation holds for both sampling periods.

Validation of the population density equations was more complex. First the variables selected in the step-wise selection procedure were used in a regression on the predictor data set. The regression equation generated was used to generate predicted values of darter density for both the predictor data set and the test data set. The predictions were then regressed against actual darter density for each period to obtain R² values with the same degrees of freedom for each. Finally, the data sets were combined and an analysis of covariance (ANCOVA) was conducted to evaluate the significance of the sampling period and the interaction between sampling period

and the model prediction.

RESULTS and DISCUSSION

Seasonality

Following data transformation, principle components were obtained for the combined data set (all sampling periods) for use in a determination of seasonal variation. Five principle components were considered significant and included as the derived independent variables. The significance criteria used was that the eigenvalue for a given component must have a value exceeding unity which indicates that the associated eigenvector explains more variation than a single variable by itself.

Once the principle components were obtained, an ANOVA was performed for each continuous variable and each component against the categorical variable Period. The seasonal variation in each variable is shown in Table 6. Seasons with the same letter are considered to be not significantly different at α =.05 level.

Table 6. Analysis of Seasonal Variation

	SAMPLING PERIOD			s	AMPLIN	G PERIC	D		
VARIABLE	JUL	ост	JAN	APR	VARIABLE	JUL	ост	JAN	APR
pН	В	С	В	Α	arcsin(vc1 ^{0.5}) _a	Α	Α	Α	Α
depth	Α	В	В,С	С	arcsin(vc2 ^{0.5}) _a	A,B	В	Α	A,B
temperature ³	Α	С	С	В	arcsin(vc3 ^{0.5}) _a	Α	Α	Α	Α
Dissolved O ₂	С	Α	A,B	В	prin comp 1	В,А	Α	С	В,С
SC ⁻³ _b	Α	С	D	В	prin comp 2	Α	В	В	В
log ₁₀ (avg vel)	A,B	Α	В	A,B	prin comp 3	Α	В	C	A
log₁₀ (bot vel)	Α	Α	Α	Α	prin comp 4	A,B	В	В	Α
log₁₀ (darter+1)	Α	В	A	Α	prin comp 5	Α	A	Α	Α

a. vc - vegetative community

b. sc - specific conductivity

Because PCA's are orthogonal (uncorrelated), experimental error elevation was not a problem.

Based on this analysis, the data set was divided into two seasons: a summer season consisting of the July and April sampling periods and a fall season consisting of the October and January sampling periods. Acknowledging that the test was inconclusive, the data could also be interpreted to indicate three seasons (summer, fall, winter) and four seasons depending on which of the variables received emphasis. Using two seasons allowed for the development of model equations with a corresponding test data set. However, one of the pitfalls of using the two season approach was that during regression analysis of the fall season, a curve fitting the October distribution of darter density would not be expected to fit the January distribution of darter density as they are not statistically the same. Nonetheless, regression analysis was performed to verify this assumption. Development of the presence/absence models would not be affected by this as they are not a function of darter density. In all subsequent analyses, the July data set was employed to develop the summer season equations with the April data set reserved for equation validation. Similarly, October was used for the fall season model generation with January as the validation data set.

Occupancy Model Development

Two models were developed for each of the seasons (summer and fall), one using the continuous transformed variables and one using principle components. In order to develop the second model, principle components were obtained for each

sampling period and added to the data sets. Following the PCA, a stepwise variable selection procedure was used to select which variables would be used in the DFA. The SAS™ default significance level for entry and retention of 0.1000 was used in the selection. The variables selected for each model are shown in Table 7. The value of Wilks' Lambda is a measure of the strength of the relationship or predictive power and is highly correlated to 1-R². Thus, the closer the value of Wilks' Lambda is to zero, the stronger the relationship or better stated the higher the percentage of explained variance. The summer season equations both explained approximately 25% of the variance. The fall season transformed continuous model explained slightly over half of the variance with the principle component model only explaining 30%. The value of Wilks' Lanmba does not translate into the number of correct or incorrect predictions the model will generate, but rather indicates the amount of seperation between groupings.

Table 7. Variables Selected for Discriminate Functions Analysis

MODEL	VARIABLES SELECTED	WILKS' LAMBDA
Summer Transformed Continuous	log bottom velocity	0.7621
Summer Principle components	principle component 1 principle component 2	0.7889
Fall Transformed Continuous	pH arcsin(vegcom1 ^{0.5}) arcsin(vegcom3 ^{0.5}) log average velocity	0.4914
Fall Principle components	principle component 1 principle component 2 principle component 3	0.6923

Once the variables were selected, they were entered into DFA's with the response variable Present which denoted presence or absence of darters. DFA develops two linear combinations of the selected variables with the goal of successfully separating the data set into two groups, present and absent. An example of the resulting equations is shown below for the summer transformed continuous variable model.

Absent = -1.71631 - 2.22288*log(bottom velocity)

Present = -3.41506 - 4.34324*log(bottom velocity)

Whichever of the two equations yields the numerically greater result for a data point is the prediction for that point.

Population Density Equations

In the development of the population density equations, four variations were tried on each sampling period. Regressions using both continuous independent variables and principle components as independent variables were run on both the whole data set and the DFA-modified data set. Recall that the DFA-modified data set consists of all locations that actually contained darters and all of the sites that were predicted to have darters (whether they did or not) according to the presence/absence model previously developed. Sites that had no darters observed and were also predicted to have no darters were removed from this data set. Table 8 summarizes the contents of each model.

Table 8. Model nomenclature and Description

Model Name	Description
Continuous Transformed Whole	Continuous transformed variables used in regression with all observations
Continuous Transformed DFA	Continuous transformed variables used in regression with only sites containing darters and those predicted to have darters by DFA
Principle Components Whole	Principle components used in regression with all observations
Principle Components DFA	Principle components used in regression with only sites containing darters and those predicted to have darters by DFA

As in the DFA, the first step in the regression analysis was to select variables. A stepwise procedure using forward selection and backwards elimination was used with the partial R^2 significance level of 0.1500. The results of the stepwise selection procedure are shown in Table 9. In all cases the dependent variable was the transformed darter density, Log_{10} (darter + 1). The use of the transformed variable was necessary to meet the normality requirements of ordinary least squares regression.

Table 9. Variables Selected for Regression Analysis

MODEL	VARIABLES SELECTED	MODEL R ²
Summer Continuous Transformed Whole	bottom velocity vegetative community 1 vegetative community 3 temperature	0.3091
Summer Continuous Transformed DFA	vegetative community 1 vegetative community 3 temperature	0.1797
Summer Principle Components Whole	principle component 2 principle component 5 principle component 1	0.2441
Summer Priciple Components DFA	principle component 5	0.08
Fall Continuous Transformed Whole	vegetative community 1 vegetative community 3 bottom velocity	0.6154
Fall Continuous Transformed DFA	vegetative community 1 bottom velocity pH dissolved oxygen depth	0.5728
Fall Principle Components Whole	principle component 1 principle component 3 principle component 4	0.3868
Fall Priciple Components DFA	principle component 1 principle component 4	0.2022

As can clearly be seen, the regressions on the whole data sets had significantly higher R² values than did those performed on the DFA modified data sets. At this point the hypothesis that the DFA modified population density equations would be more accurate than standard practice was rejected for the Comal River darter. All subsequent analyses involved only the whole data sets.

The next step in the population density equation model development was to perform regressions on the whole data sets with the variables selected above.

Although the continuous variables yielded higher predictive power than the principle component regressions, all models were retained pending validation.

Model Testing and Validation

The DFA presence/absence models were the first to be tested. The models developed for each season are shown below.

Summer Season:

Continuous Transformed Variables

Principle Components

absent =
$$-1.2 + 0.4*(prin2) + 0.3*(prin1)$$

(2)
present = $-0.6 - 0.2*(prin2) - 0.2*(prin1)$

Fall Season:

Continuous Transformed Variables

absent = -531.2+ 7.3*log(average velocity)-7.6*arcsin(vegcom3)
$$^{0.5}$$
-
2.8*arcsin(vegcom1) $^{0.5}$ +144.9*pH

(3)

present = -518.8 + 7.4*log(average velocity)-5.5*arcsin(vegcom3) $^{0.5}$ +
0.001*arcsin(vegcom1) $^{0.5}$ +143.0*pH

Principle Components

absent =
$$-0.86 + 0.2*(prin1) + 0.5*(prin2) + 0.3*(prin3)$$

(4)
present = $-0.96 - 0.2*(prin1) - 0.5*(prin2) - 0.3*(prin3)$

The presence/absence models from the DFA's were tested using a contingency table approach with the Chi-square statistic. Each model developed previously was used to generate a prediction for each grid cell for both the model data set and the corresponding test data set. The prediction was then compared to the field data for that cell. Numbers of Type I errors, Type II errors, and correct responses were tabulated for each model and placed in a contingency table as shown in Table 10. The predicted value for a particular sampling period was obtained by multiplying the total number of observations for a sampling period by the ratio of the total responses for the season to the number of total observations. For example, in Table 10 the expected number of Type I errors for July was 117*39/189 = 24.14.

The value of $X^2 = \Sigma\Sigma$ (observed - predicted)/predicted. The critical value of X^2 or a 3 x 2 contingency table is obtained by first calculating v which equals (3-1)(2-1) =2 and then looking it up in a Table of critical values. The critical value for $X^2_{2,.05} = 5.991$. The values of X^2 for each model are shown in Table 11.

Table 10. Contingency Table for Chi-Square Testing of the Occupancy Models

	PREDICTION RESULTS						
Model	TYPE I		TYPE II		CORRECT		TOTAL
Summer Continuous Transformed	observed	predicted	observed	predicted	observed	predicted	
July	19	24.14	7	7.43	91	85.43	117
April	20	14.86	5	4.57	47	52.57	72
Total	3	9	1	2	138		189
Summer Principle Components	observed	predicted	observed	predicted	observed	predicted	
July	21	24.76	5	6.81	91	85.43	117
April	19	15.24	6	4.19	47	52.57	72
Total	4	0	1	1	138		189
Fall Continuous Transformed	observed	predicted	observed	predicted	observed	predicted	
October	15	.14.20	9	16.38	65	58.42	89
January	11	11.80	21	13.62	42	48.58	74
Total	26		30		107		163
Fall Principle Components	observed	predicted	observed	predicted	observed	predicted	
October	23	21.84	6	11.47	50	50.23	89
January	17	18.16	15	9.53	42	41.77	74
Total	26		30		92		163

Table 11. X² Values for Contingency Table Testing of DFA Presence/Absence Models where H₀: The Model Performs the same between sampling periods.

	Critical Value for X ² _{2, .05} = 5.991		
MODEL	X²	TEST RESULT	
Summer Continuous Transformed	3.8944	Do Not Reject H₀	
Summer Principle Components	3.7163	Do Not Reject H₀	
Fall Continuous Transformed	9.0556	Reject H₀	
Fall Principle Components	5.878	Do Not Reject H₀	

Both the continuous transformed variable model and the principle component model for predicting darter presence/absence (equation sets 1 and 2) developed for the summer season on the July data set were acceptable for use in predicting habitat suitability for the April data set. For the fall season, only the model developed from principle components (equation set 4) from October is marginally acceptable for predicting habitat suitability in January.

Testing the regression equations developed for summer and fall with the April and January data sets respectively was the next step in the analysis. The density equations were developed by regressing the variables selected in the stepwise procedure on the log of darter density and are shown below.

Summer Season

Continuous Variables

(5) $\log_{10}(\text{darter} + 1) = 2.8 - 0.25^*(\text{bottom velocity}) + 0.008^*(\text{vegcom}1) + 0.004^*(\text{vegcom}3) - 0.10^*(\text{temperature})$

Principle Components

(6) $log_{10}(darter + 1) = 0.5 - 0.12*(prin comp 3) - 0.09*(prin comp 1) - 0.1*(prin comp 5)$

Fall Season

Continuous Variables

(7) $log_{10}(darter + 1) = 0.14 - 0.28*(bottom velocity) + 0.008(v1egcom1) + 0.004*(vegcom3)$

Principle Components

(8) $\log_{10}(\text{darter} + 1) = 0.28 - 0.07^*(\text{prin comp 1}) - 0.07^*(\text{prin comp 3}) + 0.09^*(\text{prin comp 4}) - 0.09^*(\text{prin comp 2})$

The April and July data sets were then run through the two equations generated for summer (equations 5 and 6) to generate a predicted darter density. The same procedure was accomplished for October and January with the Fall equations (7 and 8). The predictions were then regressed against observed darter densities for each sampling period using a 'no-intercept' regression model. The 'no-intercept' model was specified since the intercept was already built into the prediction. Running this regression on July and October standardized the degrees of freedom with the test sampling periods so that predictive power could be compared directly. The results of

the regressions are shown in Table 12.

Table 12. Regression Analysis Results of Predicted vs Observed Darter
Densities. DF=1

2011010100, 2.					
		R ²			
	Generation Period	Test Period	Generation Period	Test Period	
Model	July	April	October	January	
Continuous Variables	0.66	0.68	0.74	0.41	
Principle Components	0.55	0.46	0.63	0.36	

For both seasons, the continuous variable regression model yielded higher predictive power than did the corresponding principle component equation. The summer season equations appear consistent between the two sampling periods for the amount of variation explained by the model. The fall season equation, however, does not explain nearly as much of January's variation as it does for October. This is expected as the population densities are statistically different in October and January and one equation can not be expected to fit both equally well.

The final step in the determination of model validity across seasons was to conduct ANCOVA's on the combined data sets. A summer data set was built by combining the July and April data sets. Similarly, a fall data set was constructed from October and January. The ANCOVA's were run on the observed darter density versus the categorical variable 'Period', the predicted darter density, and the interaction between 'Period' and the prediction. Table 13 shows the results of the F-tests concerning each variables significance. For a model to successfully fit both sampling

periods in a season, the 'Predict' term should be significant as it indicates the regression line while the 'Period' and interaction terms should not be significant as they indicate intercept and slope modifiers respectively.

Table 13. Analysis of Covariance Results

	Variables	Summer Continuous Variables	Summer Principle Components	Fall Continuous Variables	Fall Principle Components
	Period	0.417	0.0041	0.0006	0.0003
Pr>F	Predict	0.0001	0.3557	0.0001	0.0019
	Period*predict	0.2066	0.005	0.0022	0.0096

The only model which passes α =0.05 under these constraints is the summer model with continuous variables (equation 5). All other models indicate two regression lines for the two sampling periods rather than one.

Testing for multicollinearity and normality of residuals were the final steps of the model validation process. Multicollinearity exists when there are strong relationships among the x-variables in that one or more of them may be expressed as a linear combination of the others. Multicollinearity was tested for using the collinearity diagnostics contained within SAS™ produces eigenvalues and condition indices that indicate whether multicollinearity may exist. To determine if multicollinearity exists, the condition indices (scale independent) were examined for large jumps between values. Scale independence is important as it allows for the direct comparison of variables that may have been collected in completely dissimilar measurement units. Evidence of multicollinearity exists between the vegetative

communitites and with temperature. Accomplishing the regression on the transformed x variables reduced the degree of multicollinearity but did not eliminate it. Removing temperature from the regression reduced the value of R² from 0.3156 to a value of 0.2807 and removed any traces of multicollinearity. The condition indices for each case are shown in Table 14.

Table 14. Multicollinearily Diagnostics: Condition Indicies

	Condition Index			
Variable	Continuous X's	Transformed X's	Deleted Temperature	
Intercept	1	1	1	
bottom velocity	1.75	2.12	1.45	
vegetative community 1	1.86	3.26	1.55	
vegetative community 3	3.57	4.91	3.43	
temperature	69.84	25.62		

Normality of the residuals from the regression was tested both by univariate statistical procedures previously discussed and graphically through a plot of residuals versus predicted darter density. The univariate procedure calculated a value of W = 0.985. The normal probability plot (Figure 5) was nearly perfect. The plot of residuals versus predicted darter density (Figure 6) demonstrated normality of residuals in that there existed a fairly even distribution above and below the zero axis. However, it also demonstrated mild heteroscedasticity; the value of the residuals increased with the value of the prediction. This observation was tested by regressing the predicted values of darter density on the absolute value of the regression residuals.

The regression indicated a positive slope of 0.027. The regression line is also shown in Figure 6. The degree of heteroscedasticity can be termed mild as the slope of this line is fairly flat and the model R² of 0.06 indicates a weak relationship between the magnitude of the prediction and the magnitude of the corresponding residual. Since the original regression was performed on transformed data, no options are available to eliminate this problem. While ordinary least squares regression is considered a fairly robust method, heteroscedasticity violates the assumption that errors have constant variance (Hamiltion 1992).

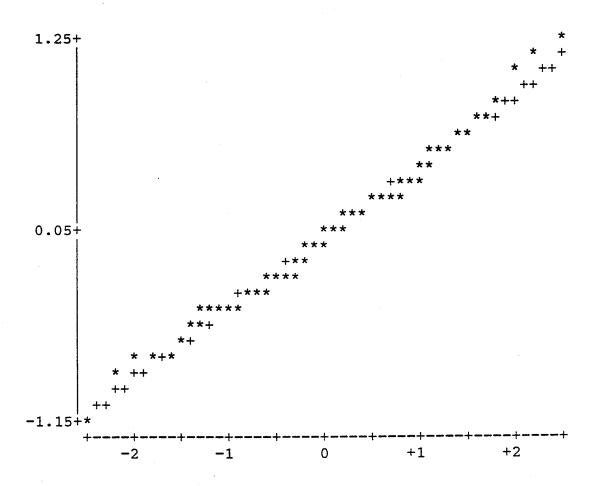


Figure 4. Normal Probability Plot of the Regression Residuals

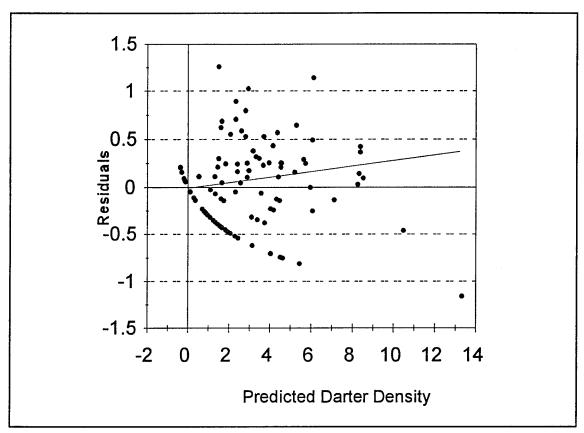


Figure 5. Plot of Regression Residuals vs Predicted Darter Density

The final version of the darter density equation for the summer season is shown below:

(9)
$$\log_{10} (darter + 1) = 0.43 - 0.3*(bottom velocity) + 0.007*(veg com 1) + 0.003*(veg com 3)$$

The predictive power of the summer season darter density equation ($R^2 = 0.28$)

indicates that this analysis failed to explain a large portion of the variation in darter densities. Similarly, the presence/absence models have fairly low (high) values of the Wilk's Lamba statistic. This indicates that the methodology employed in this study failed to account for some significant criteria for habitat selection or that factors critical to understanding habitat selection were not included in the factors analyzed. This study focused on the analysis of physical habitat variables encountered by darters living in the Comal River but did not evaluate all available data such as invertebrates, co-occurring fish species or adjacent cell vegetative characteristics. Other factors that may be equally or more important in habitat selection include predation, food supply, and competition.

One aspect of data analysis that was not addressed in this study was analysis of the spatial component. The number of data points gathered necessitated grouping the data by season rather than by location. Each cell's habitat was treated equally without regard to the habitat in adjacent cells or to that cell's location in the overall river system. In addition, this preliminary analysis did not consider stratification of the Comal River System into reaches in order to ensure adequate sample size. It is anticipated that exclusion of the strata within the new channel may improve model development.

CONCLUSIONS

1. All of the project goals were met with varying degrees of success. The statistical methodology outlined in Figure 4 provided a flexible framework to evaluate

the data set. The SAS™ programs written to accomplish this methodology are included in Appendix 3 and on the 3.5" diskette.

- 2. Three presence/absence models were successfully developed for use in determining habitat suitability for the fountain darter. Equations 1 and 2 apply to the summer season while equation 3 applies to the fall season. The equations for the summer season achieve approximately 84% accuracy while the fall season equation only achieves approximately 55% correct responses.
- 3. The two-step hierarchical approach to generating a population density predictive equation yielded significantly lower predictive power than the traditional approach of developing density equations for the whole data set. Only one population density equation was found to be statistically valid across sampling periods. Equation 9 can be used to predict darter densities during the summer season with a predictive power of approximately 28%. However, this equation suffers from heteroscedasticity and therefore violates one of the assumptions concerning linear regression.
- 4. The essential physical variables required in this analysis were velocity (both bottom and average) and vegetative community. They occur in every predictive model either directly or as substantial portions of the derived principle component.

RECOMMENDATIONS

- 1. The invertebrate data that has recently been provided should be incorporated into the data files that are the basis for this study and the analysis reaccomplished.
 - 2. A spatial component needs to be added to the analysis to account for

variation associated with distance downstream, as well as spatial effects associated with adjacent cell attributes.

- 3. The system should also be analyzed for stratification among the reaches. For example, the spring runs, new channel, and old channel are most likely statistically different rivers rather than one. This study excluded the spring run data as the difference was realized, but the new channel and old were not differentiated.
- 4. The age or size distribution of the darters needs to be accounted for in the next analysis. In this study each darter was treated as a unit fish which may not account for seasonal variation in darter concentrations due to reproduction.

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APPENDIX 1

Habitat and Flow Requirements Study for the Comal Ecosystem

Study Plan

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for

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18 September 1992

INTRODUCTION

As part of recovery implementation for listed San Marcos/Comal species and their ecosystems, as well as the conservation of candidate species, the U.S. Fish and Wildlife Service (Service) desires to assess the instream flow needs of the flow dependent aquatic resources (i.e., species and communities) of the Comal ecosystem. Although this effort will target the fountain darter (Etheostoma fonticola) and Comal Springs riffle beetle (Heterelmis comalensis), the other aquatic resources of the ecosystem are recognized as an integral part of the overall system upon which these particular species are interdependent. The Service proposes to bring together an interdisciplinary team of scientists and resource management specialists to conduct necessary studies in order to develop data and analyses that can be used to formulate instream flow strategies for the protection of the Comal ecosystem. The Service recognizes that existing and on-going research efforts within the Comal Springs ecosystem are providing valuable information to meet the study goals, and this study design is intended to complement and, where possible, integrate these efforts.

Study Objectives

To accomplish the overall study goal of developing instream flow strategies that will protect the Comal Springs ecosystem, the Service has identified the following specific study objectives:

- Objective 1: Quantify existing physical and biological characteristics of the Comal Springs ecosystem.
- Objective 2: Assess the flow dependent relationships between physical habitat and life history requirements of the aquatic species within the Comal Springs ecosystem.
- Objective 3: Develop instream flow recommendations that protect the Comal Springs ecosystem particularly native species.

To accomplish these broad objectives, several specific tasks have been identified and are discussed below.

1.0 Quantification of the Existing Physical Environment.

<u>Task 1.1</u> <u>Quantify existing and historical flow regimes.</u>

This task will involve the compilation and analysis of data on discharge rates from the Comal Springs system. This data set will be used later in the study for physical habitat modeling. Data available from the U.S. Geological Survey and others will be used to characterize discharge from all identifiable springs and water courses flowing into the Comal Springs system (Blieders Creek, Panther Canyon, and Comal Creek). Flow data will be evaluated for temporal patterns/trends (e.g., seasonal) and variation. The objective is to develop the hydrology data set to use as input for hydraulic and habitat modeling.

Task 1.2 Map existing aquatic and riparian habitats within the Comal Springs ecosystem.

A key data need for this study, as well as for long term monitoring activities, is an accurate aquatic and riparian habitat map for the entire Comal Springs ecosystem from the head water spring runs, downstream to the confluence with the Guadalupe River. Some morphometric and water course mapping data are already available and additional sources of information are being pursued. It is anticipated that airborne multi-spectral videography will be used to acquire ground coverage with a spatial resolution of no greater than 0.5 meters. This coverage may be supplemented by low level aerial photography. The imagery will be digitized and, in conjunction with ground truth data, serve as the basis to develop a GIS based (ARC/INFOTM) habitat map for the Comal Springs system. The mapping will involve the delineation of aquatic macrohabitats important to fish and other aquatic species such as runs, pools and riffles, as well as the distribution and density of aquatic and riparian vegetation. Additional map features will include the location of spring inflows, dam structures, surface water courses, water diversion structures, etc, and may be expanded based on the analysis needs of the IDT.

The mapping effort will also serve as the basis for the delineation of large scale homogeneous reaches throughout the Comal Springs ecosystem and for the development of a standardized spatial sampling protocol for the further biological characterization of the system (described below).

Task 1.3 Delineate large scale homogeneous reaches and standardized spatial sampling grid.

The sampling design to be employed is stratified random sampling. All potential sampling sites in the Comal ecosystem will be divided into homogeneous reaches (referred to below simply as reach or reaches) based on similar physical characteristics. In other words, a reach generally has homogeneity of morphometry and flow. However, each reach has spatial heterogeneity on a smaller scale in the form of different macrohabitats.

The reaches will serve as the basis for a stratified random sampling design to further characterize the biological components of the system. These reaches become the strata of the sampling design.

Based on the detailed mapping effort under Task 1.2, the entire Comal Springs system will be partitioned into reaches based on the physical characteristics. This process will be conducted by the IDT based on a review of the mapping results and site visit. The mapping effort in Task 1.2 will also provide a grid delineating macrohabitats (e.g., runs, pools, riffles, etc.) and aquatic vegetation. Multiple sites will be selected and sampled within each reach to describe the reach as a whole (Task 1.4).

Previous research by Texas Parks and Wildlife Department delineated three portions of the Comal ecosystem for their study on fountain darters, namely, Landa Lake, Comal River's old (original, natural) channel, and Comal River's new (modified) channel.

Several distinct reaches have been tentatively identified based on an initial survey and review of available data for the Comal ecosystem. Tentative reaches include the upper, middle, and lower sections of Landa Lake, spring runs (associated with springs labeled by Gunnar Brune (1981, Springs of Texas, page 131) as j, k, and l), and distinct sections of the old and new channels.

<u>Task 1.4</u> <u>Characterize the macrohabitat properties of homogeneous reaches.</u>

Once the Comal Springs system has been partitioned into discrete reaches, each reach will be examined to locate a smaller scale representative section (e.g., 100 meter longitudinal section) in which to take intensive physical measurements. Unlike the sampling design for the biological data which involved random cells within a reach, representative sections will be selected within a reach based on the distribution of macrohabitats and best professional judgment of the IDT. These smaller scale representative sections (referred to below as section(s)) will be used as prototypes of the larger scale reaches for the hydraulic modeling as described below. The sections

will be selected by the IDT based on both the mapping results and site visits. For each section, cross-section profiles of depth, velocity, substrate, vegetation type and coverage, water surface elevation and discharge will be measured at 20 to 30 locations across each macrohabitat. A sufficient number of cross-sections will be placed within each section to ensure an adequate characterization of the macrohabitat features present. Each section will be permanently marked and referenced to the map base produced in Task 1.2. If indicated, sections will correspond to (or encompass) existing transect sites used by the Texas Park and Wildlife Department.

The specific number and location of cross-sections will be determined by the IDT after a review of the mapping results and site visits. Measurements at each cross-section will be taken at a minimum of three discharge levels and if possible, given time, manpower and flow constraints, as many additional data sets will be taken as practical.

Flow levels targeted for measurement are at approximately each 50 percent reduction in flow over discharge ranges between 500 and 0 cubic feet/second as measured at the USGS's gage near the San Antonio Street Bridge.

Task 1.5 Develop and test hydraulic models appropriate for use in simulating characteristics of macrohabitats within each homogeneous reaches.

This task will focus on the development and testing of suitable hydraulic models to represent each of the study sites where intensive data have been collected under Task 1.4. The hydraulic models will be used to estimate macrohabitat hydraulic properties such as water surface elevations, depth, and velocities over ranges of discharges not measured during the field investigations. Selection and application of models will be determined after the initial field data collections conducted under Task 1.4 and reviewed by members of the IDT. It is anticipated that Landa Lake and areas significantly influenced by back water effects from dams will be modeled using fast flushing (small) reservoir models, while the more typical flowing stream habitats will be modeled using hydraulic simulations routines developed by the Service's Instream

Task 1.6 Evaluate and use aquifer models to generate synthetic flow regime dataset

A number of relations and models have been developed that relate aquifer levels to discharge rates of the Comal Springs system. The Interdisciplinary Team (IDT) will review these models and select the most suitable model(s) to generate summary results that relate seasonal aquifer levels to seasonal spring discharge rates. The specific content and format of the model output will be identified based on the needs of modeling efforts by IDT members.

2.0 Quantification of the Existing Biological Environment.

Task 2.1 Document historical abundance of key/target aquatic and riparian system species.

Considerable effort has been invested by several entities to delineate the distribution and abundance of aquatic and riparian species within the Comal Springs ecosystem. Recent work is now available which will add significantly to the understanding of the ecosystem. This task will focus on obtaining and summarizing available information for use in the existing study, especially for those efforts that have quantitative data on both species abundance, distribution, and macrohabitat use.

Task 2.2 Determine existing distribution of aquatic species within specific macrohabitats in each large scale homogeneous reach.

For each of the reaches, the surface area will be partitioned into 10 m² (3.16 m by 3.16 m) grid to produce a series of unique cells that define the available macrohabitats throughout each reach (stratum). Texas Parks and Wildlife Department's work on the Comal involved sampling 10 m² cells and the intent is to use a similar size cell. Within each reach, a minimum of 10 percent of the cells will then be randomly selected for use in the biological sampling efforts described below. Randomly

selected cells will then be further divided into 0.5 m² (70.7 cm by 70.7 cm) sub-cells for use in the macroinvertebrate sampling efforts as described below. The grid system(s) and data developed for each reach will be added to the GIS map system. Biological sampling will be conducted within a one week period of the hydraulic sampling efforts, or in the event that flows do not change more than 50 percent over a three month period, the biological sampling will be initiated.

Landa Lake/Impoundment Aquatic Macroinvertebrates: All selected sub-cells within each reach of Landa Lake and other non-flowing reaches within the Comal Springs system will be sampled for snail population densities by randomly selecting three 0.5 m² areas within each 10 m² cell to estimate snail densities using the existing methodology employed by Dr. Tom Arsuffi. This technique uses a plexiglass viewing plate of 0.5 m² dimensions held at the water surface. The vegetation is illuminated using hand held diving lights and the numbers and, where feasible, the species of snails will be enumerated within each of the selected three sampling locations. Snail sampling will be accomplished during the evening period when snails migrate to the top of the vegetation. At each of the sampled cells, the depth, velocity, substrate, and vegetation type, including percent aerial coverage and height above the bottom will be noted at the time of sampling. Water temperature, turbidity (or transparency), conductivity, dissolved oxygen, and pH will also be measured in each sampled cell during the field collections. In addition to the snail density estimates within each sub-cell, other aquatic macroinvertebrate densities will be estimated using a suitable sampling protocol. At present, the specific sampling protocol has not been determined by the IDT and will be decided after review of previous sampling success identified under Task 2.1. This sampling effort would be conducted immediately after completion of the three replicate snail density estimates within each cell, within the same three replicate sub-cell sites.

Riverine Aquatic Macroinvertebrates: Sampling of riverine reaches will also involve the random selection of a minimum of 10 percent of the available area within each reach. For each of the selected cells, 3 replicate samples will be taken from randomly

selected sub-cells within the 10 m² cell. Sampling within the spring runs will follow the existing techniques of Dr. Cheryl Barr for the Comal Springs riffle beetle. Sampling for aquatic macroinvertebrates in other riverine areas will employ kick net samples or other sampling gear identified by the IDT after a review of previous sampling efforts identified under Task 2.1. At each of the sampled cells, the depth, velocity, substrate, and vegetation type, including percent aerial coverage and height above the bottom will be noted at the time of sampling. Water temperature, turbidity, transparency, conductivity, dissolved oxygen, and pH will also be measured in each sampled cell during the field collections.

Fisheries: Fish distribution and abundance using a combination of netting, electrofishing, trapping, and visual gear will be conducted within each randomly selected 10 m² cell within each reach. Sampling for fishes will be conducted between 24 and 48 hours after the aquatic macroinvertebrate sampling. The specific sampling strategy for a selected cell will be determined at the time of sampling based on the depth, vegetation cover, and other physical factors that may severely bias the sampling effort. The total number of each species of fish observed or captured in each cell will be noted, including either visual estimates of the standard length (mm) measured for captured individuals. If age class (i.e., Ø class, I class) is apparent, that will be noted. At each of the sampled cells, the depth, velocity, substrate, and vegetation type, including percent aerial coverage and height above the bottom will be noted at the time of sampling. Water temperature, turbidity (or transparency), conductivity, dissolved oxygen, and pH will also be measured in each sampled cell during the field collections.

Zooplankton of Comal Springs Ecosystem

Zooplankton abundance and distributions will be evaluated using a conventional methods.

Task 2.3 Summarize life history requirements of the aquatic system species, especially those most likely to be flow dependent at the microhabitat scale.

Many of the native and non-native species have been studied extensively (e.g., smallmouth bass) while others have received little or no extensive life history work. The existing literature and unpublished data on the life history requirements of the identified aquatic species will be solicited from management agencies and researchers active in the Comal Springs system, San Marcos River system, and other systems containing the more ubiquitous species such as the non-native fishes. This information will be supplemented by the collection efforts outlined in Task 2.2. Compilation of this material will aid the IDT to select target species for inclusion in the instream flow analyses based on the extent and applicability of available literature/data in conjunction with the information collected during the biological sampling efforts. The selection of target species for inclusion in the instream flow assessments will be given to both native and non-native species of fish, invertebrates, and vegetation with a goal of representing the diversity of species habitat requirements or community level assemblages. Final selection of target species may to a large degree be determined by lack of suitable data or knowledge of life history requirements.

Task 2.4 Develop habitat suitability functions for target native and non-native aquatic species for use in habitat modeling.

This task will involve a synthesis of the life history information collected on both the native and non-native species, the sampling data obtained from the biological sampling efforts, and professional judgement of knowledgeable researchers to develop habitat suitability functions. These functions will serve as the basis for modeling habitat requirements over the range of discharges to be examined in this study. The form of the suitability criteria will be determined based on the availability of existing data and that data collected during the biological field sampling efforts. It is likely that many of the target species will not have sufficient quantitative data or known life history requirements and therefore a guilding approach may be required. If guilding is undertaken, then representative species from each of the spatial niches (i.e.,

macrohabitats) will be selected with the best quantitative data and collective knowledge of species experts to represent species for which habitat suitability data are not available. Based on the initial review of available information on species life history requirements and data analyses from the biological sampling program, laboratory studies may be initiated by the IDT for selected species to obtain necessary data judged to be critical to the successful determination of flow dependent relationships used in the instream flow assessment process.

This task will bring together all available data on the life history and habitat associations of fountain darters with the intent of formulating an accurate and unbiased suitability criteria.

3.0 Analyze Flow Dependent Relationships of Target Native and Non-native Aquatic and Riparian System Species.

Task 3.1 Develop flow dependent relationships for target native and non-native species for each homogeneous reach within the system.

The habitat suitability functions for the native and non-native target species developed under Task 2.4 will be used in conjunction with the hydrology and hydraulic model outputs developed under Tasks 1.1 and 1.4 to produce flow dependent relationships for available habitat conditions throughout the Comal Springs system for the range of simulated discharges. In addition, other modeling approaches will be considered, such as spatial niche diversity as a function of discharge, Habitat Suitability Index Models (HSI), Habitat Evaluation Procedures (HEP), Index of Biotic Integrity (IBI), etc, based on on-going data analysis and discussions of the IDT. Spatial niche diversity analyses, for example, would focus on a determination of the relationship between the number and characteristics of specific macrohabitat types within each reach as a function of flow magnitude and flow magnitudes that maximize the diversity of these features would be considered in light of the habitat requirements of the target native and non-native species. Modeling approaches will be discussed, examined and applied where such techniques improve the understanding of the interaction between

flow and life history requirements of the native and non-native target species within the Comal Springs ecosystem. Although physical habitat modeling will be the primary focus of this effort, water quality modeling may be initiated based on the general consensus of the IDT, that water quality may become limiting within Landa Lake during very low discharge periods. The decision to undertake water quality modeling will be made based on a review of the available data, data collected during the biological sampling efforts, time, and budgetary constraints.

Task 3.2 Formulate instream flow recommendations that optimize for native species and minimize conditions for non-native species.

The results of the various modeling efforts, literature reviews, data analyses, and professional judgment of knowledgeable species experts will then be integrated by the IDT to develop instream flow recommendations that optimize the protection of native species within the Comal Springs system while minimizing, where possible, favorable conditions for non-native species. It is recognized that some non-native species may be eurytopic, having a wide range of tolerance to a number of environmental factors, including, but not limited to flow. However, some non-natives (e.g., the herbivorous giant (Columbian) rams-horn snail, Marisa cornuarietis) may be at a disadvantage during certain flow regimes (i.e, under suboptimal or unsuitable conditions). It is fitting and important to know what minimizes suitable conditions for non-natives and take that in to consideration when developing recommendations for to optimize habitats for native species.

It is anticipated that a range of flows will be considered, possibly on a seasonal basis, where flow magnitudes may result in anticipated unacceptable adverse impacts to native, particularly listed, species.

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Time required table by task

(Exclusive of Dr. Thomas B. Hardy's time).

Technician refers to any competent individual with the background to help in subject area IDT refers to collective study team (independent review or as a team meeting by context) Other disciplines by titles

Task 1.1	Hydrologist	week review [USGS DATA] time per model week processing time and summarization of information
	Technician IDT	4 week support for hydrologist 2 days review of model choice and model output review
Task 1.2	Technician	weeks x 2 people ground truth mapping - vegetation and fisheries background week existing data compilation
Task 1.3	Technician IDT	 1 week grid delineation and GIS update. 3 days review of reach delineations and <u>collective</u> site visit if necessary
Task 1.4	Technician	1 week x 8 people per flow level help with cross section surveys
	IDT	1 week select representative sections in each reach and cross section location
Task 1.5	IDT	3 days review of hydraulic model selection by reach
Task 1.6	Hydrologist	
Task 2.1	Technician IDT	2 weeks data summary of requested information 3 days review of existing information
Task 2.2	Technician	1 week grid development and random cell generation1 week x 12 people each flow or 3 month period invert and fish collection
		1 month for aquatic invert sample processing if

undertaken for each sample period

	ries tebrate Ecology tic Vegetation	2 weeks data reduction and analysis 1 week data analysis per sampling effort 1 week data analysis per sampling effort 1 week data analysis per sampling effort 3 day summary review each trip
Task 2.3	Technician IDT IDT	3 weeks data request and data summary 1 week for each taxa and/or species expert 2 days target species selection
Task 2.4	Technician IDT	1 week data analysis and support2 weeks data review, discussion, etc.
Task 3.1	Technician IDT	2 months analysis support 1 month data/analysis review
Task 3.2	Technician IDT	1 month report generation support 1 month analysis review, integration, discussion, report writing

Timing of Task Start and Completion

	Start	End
Task 1.1	Immediately	End of 1st month of project
Task 1.2	Late September 1992	October 31, 1992
Task 1.3	October 15, 1992	October 31, 1992
Task 1.4	November 1, 1992	3 months before report due
Task 1.5	Immediately	January 1993
Task 2.1	Immediately	January 1993
Task 2.2	November 1, 1992	3 months before report due
Task 2.3	Immediately	April 1993
Task 2.4	December 1992	3 months before report due
Task 3.1	April 1993	3 months before report due
Task 3.2	June 1993	1 month before report due
Draft Repor	rt .	August 31, 1993
Report Rev	iew and Revisions	
Final Repor	t	October 29, 1993

APPENDIX 2

This appendix includes information on the structure of the data files used in accomplishing the Comal River project, codes for interpreting categorical data, and the SAS printout of the combined data set.

File Structure

Quattro Pro Files

Comal.wb1-contains the raw physical habitat data for July and October

Comal2.wb1-contains the raw physical habitat data for January and April

Fish1a.wb1-contains the fish sampling data for July

Fish1b.wb1-contains the fish sampling data for October

Fish2a.wb1-contains the fish sampling data for January

Fish2b.wb1-contains the fish sampling data for April

Sample1f.wb1-combines the physical and fish data for July, calculates average velocities

Sample2f.wb1-same as above but for October

Sample3f.wb1-same as above but for January

Sample4f.wb1-same as above but for April

April.txt-ASCI text files of selected fields from Sample4.wb1-allows export to SAS

Jan.txt-ASCI text files of selected fields from Sample3.wb1-allows export to SAS

July.txt-ASCI text files of selected fields from Sample1.wb1-allows export to SAS

Oct.txt-ASCI text files of selected fields from Sample2.wb1-allows export to SAS

SAS files

April.dat-SAS conversion of April.txt downloaded from Quattro Pro. Data files also exist for the other three text files; same name, new extension.

A printout of the combined data set, all sampling periods, is included in this Appendix.

The variables used are explained below:

Obs-SAS observation number starting with n=1.

Strata-The strata the sample was taken from.

Grid - The grid number within the strata of the cell sampled.

Perveg- percent vegetative cover

Domveg - Dominant vegetative cover species - see USF&WS attachment for codes

Subveg - Subdominant vegetative cover species

Perdv - percent dominant vegetative species = .75*perveg if subveg exists, else = perveg

Persv - percent subdominant vegetation = .25*perveg

Temp - water temperature

DO - dissolved oxygen content

pH - pH of the sample

SC - specific conductivity

Depth - depth of the sample cell

VBOT - velocity 15 cm from the cell bottom

VAVG - mean column velocity

NUMSPEC - number of fish species caught

NUMPASS - number of sampling passes for fish

Darter - number of darters caught

Period - indicates month sample taken: 1 = July, 2=October, 3=Jan, 4=April

DVEGCLS - dominant vegetative cover class (1-3)

SVEGCLS - subdominant vegetative class (1-3)

DCOM1- percent of dominant vegetation that is type 1 in a grid cell

DCOM2 & DCOM3 - same as DCOM1 except for types 2 & 3 veg classes

SCOM1 - SCOM3 - same as DCOMs except for subdominant vegetation

VEGCOM1-Overall vegetative community type 1 for a cell = DCOM1 + SCOM1

VEGCOM2 & VEGCOM3- same as vegcom1 except for types 2 & 3

Note: Sum of 3 VEGCOM variables equals the percent vegetative cover for a cell.

The codes used by the USF&WS for substrates, plants, habitat types, fish, and invertebrates is also included in this Appendix.

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		0.180 1.455 0.620 1.330 0.280 0.440 0.680 0.030 0.040 0.010 0.010 0.060 0.130 0.225 0.520	V A V G

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307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324	1 0 6 4 5 1 2 3 6 1 4 4 1 5 1 4 1 4 1 4 1 4 1 4 1 4 1 4 4 1 4 1	15 15 15 15 15	4 0 7 5 1 0 3	4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	3 3 · · · · · · · · · · · · · · · · · ·		0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0	0 0 0 0 0 0 0	80 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0			0. 0. 0. 52. 52. 18. 35. 0. 25. 0. 18. 0. 70. 52. 75.	0055800000800005	0. 0. 0. 0. 0. 0. 0. 0. 0. 17	000000000000000000000000000000000000000	0 0 0 0 0 0 0 0 0 0 0 0 0	0 0 17 17 6 0 0 0 0 0 0 0 0 0 0 0		1 2	0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0	0.0 80.0 80.0 0.0 0.0 0.0 0.0 0.0 0.0 0.	0.0 0.0 70.0 70.0 25.1 35.0 0.0 25.0 0.0 18.8 0.0 60.0 70.0 52.5 75.0	

325 326 327 328 329 330 331 332 333 334 335 336 337 338 340 341 342	O B S	325 326 327 328 329 330 331 332 333 334 335 336 337 338 340 341 342	O B S
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3 3 1 3 1 1 3 1 3 1	D V E G C L S	00 95 00 00 00 00 00 00 00 00 00 00 00 00 00	P E R V E G
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		0.005 0.055 0.055 0.040 0.015 0.140 0.100 0.060 0.020 	V A V G

O B S	S T R A T A	G F I	₹		P E R V E G	D O M V E G	S U B V E G		P E R D		P E R S V		T E M P	I ()	P H	s C	D E P T H	V B O T	V A V G
343 344 345 346 347 348 349 350 351 352	8 8 8 8 8 9 9 9 9 9	33 33 44 22 33	190 247 341 39 391 496 210 248 311	2 9 1 4	5 0 0 5 5 0 0 0 0	5 0 0 17 30 5 0 1 0	0 0 0 0 7 0 14 0	2 6 1	35.0 0.0 0.0 25.0 5.0 57.5 10.0 30.0 0.0	2:	0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0	24 23 24 23 24 23 23 23		7 7 8 8 8 5 5 5	. 56 . 87 . 21 . 85 . 15 . 25 . 24 . 30	7.60 7.60 7.70 7.60 7.60 7.60 7.50 7.50 7.50	2 550 5 544 5 547 5 547 7 552 7 552	2.40 0.80 1.40 1.30 1.80 2.1.20 1.70 2.0.40	0.03 0.04 0.30 0.22 0.02	0.015 0.050 0.300 0.220 0.050 0.040 0.300 0.080 0.060
O B S	N U M S P E C	N U M P A S	T E	P E R I	С	V E G C L		D C O M 1	I ((N			D C O M 3	C O M	S C O M 2		S C O M 3	V E G C O M 1	V E G C O M 2	V E G C O M 3	
343 344 345 346 347 348 349 350 351 352	3 1 2 4 3 6 2 2 2 2	15 15 15 15 14 15 15 15	4 0 3 10 6 8 0 0 0	4 4 4 4 4 4	2 • 3 1 2 • 3 • 3		0 . 0 . 5 . 0 .	0 0 0 0 0 0 0 0 0	85.0 0.0 0.0 0.0 67.5 0.0 0.0		0. 0. 25. 0. 0. 30.	0 0 0 0 0 0	0.0 0.0 0.0 22.5 0.0	0 0 0 0 0 0	0 . 0 . 0 . 0 . 10 .	.0 .0 .0 .0	0.0 0.0 0.0 5.0	85.0 0.0 0.0 0.0 0.0 67.5 0.0 0.0	0.0 0.0 25.0 0.0 0.0 40.0 0.0	

SUBSTRATE

What I would like to do is recode using the modified Wentworth Scale Substrate Classification and place our observations as best we can into the following:

COI	E DE Classification		Include old codes
Ø	ORGANICS	MISC. ORGAN. DEBRI	S 11, 15, 17, 18
1	CLAY	< 0.004	10
2	SILT	0.004 - 0.062	1, 7, 13, 16, 19, 21, 24
3	SAND	0.062 - 1	2
4	COARSE SAND	1 - 2	
5	GRANULE	2 - 4	$\overline{20}$
6	SM. GRAVEL	4 - 8	8
7	MED. GRAVEL	8 - 16	3, 23
8	LG. GRAVEL	16 - 32	<u></u>
9	RUBBLE	32 - 64	
10	SM. COBBLE	64 - 128	4, 5
11	LG. COBBLE	128 - 256	<u> </u>
12	SM. BOULDER	256 - 512	6, 22
13	MED. BOULDE	R 512 - 1024	<u>_</u>
14	LG. BOULDER	> 1024	
15	BEDROCK	SOLID SUBSTRATE	12, 14

None of the following substrate codes should be used:

16, 17, 18, 19, 20, 21, 22, 23, 24

I place plant debris, leaf litter, and misc. plant detritus in #0 = organics.

I place mud, fines, in # 2 = silt.

I place all sand in #3 = sand.

I place gravel in # 7 = med. gravel (unless more specific info. was recorded).

I place cobble in # 10 = small cobble

I place all boulders in # 12 = small boulders

I place concrete in # 15 = bedrock, (I believe all references on data sheet refer to smooth concrete (Comal section 10 comes to mind). If it was clastic, it should have been recorded in the field as boulders or whatever size)

In the future, forms should be filled out with the modified Wentworth.

HABITAT TYPES

mod = moderate.

THE

CODE HABITAT TYPE

- 1 backwater
- 2 run
- 3 eddy
- 4 fast run
- 5 slow run
- 6 back eddy
- 7 moderate run
- 8 edge
- 9 riffle
- 10 pool
- 11 side channel
- 12 fast, clear water [probably fast run]
- 13 springrun

Plants and Algae

8-character

18

19

24-

30-

myriophy

hydrocot

amblyste

algaespp

18

19, 49

24, 29

7	THE scientif	OLD	DESCRIPTION OF
	CODE DATACO	ODE CODE(S)	VEGETATION
	l' ludwigia charaspp ricciafl egeriade vallisne nupharlu rhyzoclo cabombac bryophyt utricula ceratopt hydrilla potamoge	1, 22, 42 2 3, 54 4 5, 34, 36 6, 20, 23, 26, 47 7, 27 8, 21 9, 48 10	water primrose, <u>Ludwigia repens</u> chara species riccia, <u>Riccia fluitans</u> waterweed, <u>Egeria densa</u> wild celery, <u>Vallisneria americana</u> yellow cow lily, <u>Nuphar luteum</u> filamentous algae, <u>Rhyzoclonium</u> sp. et al. fanwort, <u>Cabomba caroliniana</u> unidentified moss, bryophyte bladderwort, <u>Utricularia gibba</u> water sprite, <u>Ceratopteris thalictroides</u> hydrilla, <u>Hydrilla verticillata</u> pondweed, <u>Potamogeton sp.</u>
1	6' sagittar	16, 39, 58	cat-tail, <u>Typha</u> sp. delta arrow-head, <u>Sagittaria platyphyla</u>
		17, 45	water willow, Justicia americana

30, 33, 50, 52, 56 algae, various species, usu. attached 31eleochar 31, 40, 53 spikerush, Eleocharis sp.

32 nasturti

watercress, N. officinale (= Rorippa n-aquaticum) 32 37

water-milfoil Myriophyllum brasiliense et al.

water-pennywort, Hydrocotyle sp.

amblystegium, Amblystegium riparium

grassspp 37 unidentified grass

41 colocasi 41, 46 elephant ear, Colocasia esculenta

51polygonu 51 smartweed, Polygonum spp.

55 irissspp 55 unidentified iris/flag

None of the following plant codes should be used: 33, 40, 50, 52, 56, 57, 60

(3) Fishes and Invertebrates;

THE CODE	FISH/invertebrate NAME	OLD CODE(s)	
1	FOUNTAIN DARTER		
2	UNIDENTIFIED GAMBUSIA	1	
3	LARGESPRING GAMBUSIA	2, 30	
4	MOSQUITOFISH	3 4	
5	SAILFIN MOLLY	•	
6 .	ROCK BASS	5	
7	SPOTTED SUNFISH	6 7	
8	ROUNDNOSE MINNOW		
9	LARGEMOUTH BASS	8, 24	
10	UNIDENTIFIED SUNFISH SP.	9	
11	RIO GRANDE CICHLID	10, 15, 16, 17, 36, 60	
12	GREENTHROAT DARTER	11, 41	
13	MEXICAN TETRA	12	
14	WARMOUTH	13	
18	UNIDENTIFIED ETHEOSTOMA	14	
19	YELLOW BULLHEAD	18, 49	
20	EURYCEA SP.	19	
21	CRAYFISH	20	
22	ODONATE NYMPHS	21, 42, 43, 46	
23	GREEN SUNFISH	22 23	
25	BLUEGILL	25	
26	LONGEAR SUNFISH	26	
27	TEXAS SHINER	27	
28	MIMIC SHINER	28	
29	REDBREAST SUNFISH	•	
31	UNIDENTIFIED FISH	29, 60	
32	UNIDENTIFIED MINNOW	31, 61	
33	UNIDENTIFIED MICROPTERIS SP.	32, 47, 52, 54	
34	UNIDENTIFIED CICHLID SP.	• -	
35	TADPOLE SI	34 35	
		35	

AE CODE	EICU/:	OLD	
/	FISH/invertebrate NAME	CODE(s)	
37	GUADALUPE BASS	37	
38	REDEAR SUNFISH	38	
39	ORANGETHROAT DARTER	39	•
40	WEED SHINER	40	
44	GLASS SHRIMP	44	
45	TURTLE	45	
48	BIVALVES	48	
50	GIANT RAMSHORN SNAIL	50	
51	BLACK BULLHEAD	51	
53	SMALLMOUTH BASS	53	. •
5 6	UNIDENTIFIED BULLHEAD SP.	56	
<i>5</i> 7	STINKPOT	57	•
59	UNIDENTIFIED TILAPIA SP.	59	
62	GIANT RAMSHORN SNAIL EGGS	62, 64	
63	UNIDENTIFIED CATFISH	63	
66	THIARA GRANIFERA		
67	THIARA TUBERCULATA		
		•	

None of the following fish/invertebrate codes should be used: 15, 16, 17, 24, 30, 36, 41, 42, 43, 46, 47, 49, 52, 54, 55, 58, 60, 61, 64, 65.

THERE SHOULD BE NO SAMPLES WITH

	fish name	old code
Menidia beryllina	MENDIA - B redthroat sunfish	55 60
	brown bullhead	65

Please identify Section, cell, and date of sample if these pop up.

APPENDIX 3

PERIOD.SAS

```
libname lib 'c:\saswork';
data lib.july;
infile 'c:\saswork\july.dat' pad;
input strata$ 1-5 grid$ 9-15 perveg 17-19 domveg 25-26 SUBVEG 33-34
PERDV 41-46 PERSV 49-53 TEMP 57-62 DO 65-70 PH 73-76 SC 81-85 DEPTH 89-93
VBOT 97-101 VAVG 105-109 numspec 113-114 numpass 121-122 darter 129-130;
run:
data july; set lib.july;
period=1;
run;
libname lib 'c:\saswork';
data lib.OCT;
infile 'c:\saswork\oct.dat' pad;
input strata$ 1-5 grid$ 9-15 perveg 17-19 domveg 25-26 SUBVEG 33-34
PERDV 41-46 PERSV 49-53 TEMP 57-62 DO 65-70 PH 73-76 SC 81-85 DEPTH 89-93
VBOT 97-101 VAVG 105-109 numspec 113-114 numpass 121-122 darter 129-130;
run;
data oct; set lib.oct;
period=2;
run;
libname lib 'c:\saswork';
data lib.jan;
infile 'c:\saswork\jan.dat' pad;
input strata$ 1-5 grid$ 9-15 perveg 17-19 domveg 25-26 SUBVEG 33-34
PERDV 41-46 PERSV 49-53 TEMP 57-62 DO 65-70 PH 73-76 SC 81-85 DEPTH 89-93
VBOT 97-101 VAVG 105-109 numspec 113-114 numpass 121-122 darter 129-130;
run;
data jan; set lib.jan;
period=3;
run:
libname lib 'c:\saswork';
data lib.april;
infile 'c:\saswork\april.dat' pad;
input strata$ 1-5 grid$ 9-15 perveg 17-19 domveg 25-26 SUBVEG 33-34
PERDV 41-46 PERSV 49-53 TEMP 57-62 DO 65-70 PH 73-76 SC 81-85 DEPTH 89-93
VBOT 97-101 VAVG 105-109 numspec 113-114 numpass 121-122 darter 129-130;
run;
```

```
data april; set lib.april;
period=4;
run;
data all;
set july oct jan april;
run;
data vegcls; set all;
if domveg=1 then dvegcls=3;
if domveg=2 then dvegcls=3;
if domveg=3 then dvegcls=1;
if domveg=5 then dvegcls=2;
if domveg=6 then dvegcls=3;
if domveg=7 then dvegcls=1;
if domveg=8 then dvegcls=1;
if domveg=9 then dvegcls=1;
if domveg=10 then dvegcls=1;
if domveg=12 then dvegcls=3;
if domveg=14 then dvegcls=3;
if domveg=16 then dvegcls=2;
if domveg=17 then dvegcls=3;
if domveg=19 then dvegcls=3;
if domveg=24 then dvegcls=1;
if domveg=30 then dvegcls=1;
if domveg=31 then dvegcls=3;
if domveg=32 then dvegcls=3;
if domveg=51 then dvegcls=3;
if subveg=1 then svegcls=3;
if subveg=2 then svegcls=3;
if subveg=3 then svegcls=1;
if subveg=5 then svegcls=2;
if subveg=6 then svegcls=3;
if subveg=7 then svegcls=1;
if subveg=8 then svegcls=1;
if subveg=9 then svegcls=1;
if subveg=10 then svegcls=1;
if subveg=12 then svegcls=3;
if subveg=14 then svegcls=3;
if subveg=16 then svegcls=2;
if subveg=17 then svegcls=3;
if subveg=19 then svegcls=3;
if subveg=24 then svegcls=1;
if subveg=30 then svegcls=1;
if subveg=31 then svegcls=3;
```

```
if subveg=32 then svegcls=3;
if subveg=37 then svegcls=3;
if subveg=41 then svegcls=3;
if subveg=51 then svegcls=3;
if subveg=55 then svegcls=3;
run;
data percls; set vegcls;
if dvegcls=1 then dcom1=perdv;
else dcom1=0:
if dvegcls=2 then dcom2=perdv;
else dcom2=0;
if dvegcls=3 then dcom3=perdv;
else dcom3=0;
if svegcls=1 then scom1=persv;
else scom1=0;
if svegcls=2 then scom2=persv;
else scom2=0;
if svegcls=3 then scom3=persv;
else scom3=0;
run;
data lib.final; set percls;
vegcom1=dcom1+scom1;
vegcom2=dcom2+scom2;
vegcom3=dcom3+scom3;
run;
data lib.final; set lib.final;
 if strata=2 then delete;
 if strata=6 then delete;
proc print;
run;
proc univariate data=lib.final plot;
var ph depth temp do sc vbot vavg darter vegcom1 vegcom2 vegcom3;
run;
```

NORM.SAS

```
libname lib 'c:\saswork';
data work1; set lib.final;
if darter=0 then present=0;
else present=1;
run;
data july; set work1;
if period=1;
run;
data oct; set work1;
if period=2;
run;
data jan; set work1;
if period=3;
run;
data april; set work1;
if period=4;
run;
data july2; set july;
tempcube=temp**3;
logdo=log10(do);
ncubesc=-sc**-3;
logvnos=log10(vbot);
logvavg=log10(vavg);
rtvc1=sqrt((vegcom1)/100);
rtvc2=sqrt((vegcom2)/100);
rtvc3=sqrt((vegcom3)/100);
run;
data july3; set july2;
tranvc1=arsin(rtvc1);
tranvc2=arsin(rtvc2);
tranvc3=arsin(rtvc3);
run;
data oct2; set oct;
```

tempcube=temp**3;

```
logdo=log10(do);
ncubesc=-sc**-3;
logvnos=log10(vbot);
logvavg=log10(vavg);
rtvc1=sqrt((vegcom1)/100);
rtvc2=sqrt((vegcom2)/100);
rtvc3=sqrt((vegcom3)/100);
run;
data oct3; set oct2;
tranvc1=arsin(rtvc1);
tranvc2=arsin(rtvc2);
tranvc3=arsin(rtvc3);
run;
data jan2; set jan;
tempcube=temp**3;
logdo=log10(do);
ncubesc=-sc**-3;
logvnos=log10(vbot);
logvavg=log10(vavg);
rtvc1=sqrt((vegcom1)/100);
rtvc2=sqrt((vegcom2)/100);
rtvc3=sqrt((vegcom3)/100);
run;
data jan3; set jan2;
tranvc1=arsin(rtvc1);
tranvc2=arsin(rtvc2);
tranvc3=arsin(rtvc3);
run;
data april2; set april;
tempcube=temp**3;
logdo=log10(do);
ncubesc=-sc**-3;
logvnos=log10(vbot);
logvavg=log10(vavg);
rtvc1=sqrt((vegcom1)/100);
rtvc2=sqrt((vegcom2)/100);
rtvc3=sqrt((vegcom3)/100);
run;
data april3; set april2;
tranvc1=arsin(rtvc1);
```

```
tranvc2=arsin(rtvc2);
tranvc3=arsin(rtvc3);
run:
data work2; set work1;
ncubesc=-sc**-3:
tempcube=temp**3;
logdo=log10(do);
logvnos=log10(vbot);
logvavg=log10(vavg);
rtvc1=sqrt((vegcom1)/100);
rtvc2=sqrt((vegcom2)/100);
rtvc3=sqrt((vegcom3)/100);
run;
data lib.tcomal; set work2;
tranvc1=arsin(rtvc1);
tranvc2=arsin(rtvc2);
tranvc3=arsin(rtvc3);
run;
proc univariate data=july3 normal plot;
var ph depth tranvc1 tranvc2 tranvc3 tempcube logdo
ncubesc logvnos logvavg;
run;
proc univariate data=oct3 normal plot;
var ph depth tranvc1 tranvc2 tranvc3 tempcube logdo
ncubesc logvnos logvavg;
run;
proc univariate data=jan3 normal plot;
var ph depth tranvc1 tranvc2 tranvc3 tempcube logdo
ncubesc logvnos logvavg;
run;
proc univariate data=april3 normal plot;
var ph depth tranvc1 tranvc2 tranvc3 tempcube logdo
ncubesc logvnos logvavg;
run;
proc univariate data=lib.tcomal normal plot;
var ph depth tranvc1 tranvc2 tranvc3 tempcube logdo
```

ncubesc logvnos logvavg; run;

DISCRIM.SAS

```
libname lib 'c:\saswork';
proc princomp data=lib.tcomal out=out outstat=stat n=5;
var ph depth tempcube logdo ncubesc logvnos logvavg tranvc1
tranvc2 tranvc3;
run;
proc print data=stat;
run;
proc glm data=out;
class period;
model prin1 prin2 prin3 prin4 prin5=period;
means period/regwq;
run;
proc glm data=lib.tcomal;
class period;
model ph depth tempcube logdo ncubesc logvnos logvavg tranvc1
tranvc2 tranvc3=period;
means period/regwq;
run;
proc princomp data=lib.tcomal out=prin outstat=stat n=5;
var ph depth tempcube logdo ncubesc logvnos logvavg tranvc1
tranvc2 tranvc3;
by period;
run;
proc print data=stat;
run;
data lib.prinjul; set prin;
if period=1;
run;
data lib.prinoct; set prin;
if period=2;
run;
data lib.prinjan; set prin;
if period=3;
run;
data lib.prinapr; set prin;
```

```
if period=4;
run;
proc stepdisc data=lib.prinjul slentry=0.10;
var ph depth tempcube logdo ncubesc logvavg logvnos tranvc1 tranvc2 tranvc3;
class present;
run;
proc stepdisc data=lib.prinoct slentry=0.10;
var ph depth tempcube logdo ncubesc logvavg logvnos tranvc1 tranvc2 tranvc3;
class present;
run;
proc stepdisc data=lib.prinjul slentry=0.10;
var prin1 prin2 prin3 prin4 prin5;
class present;
run;
proc stepdisc data=lib.prinoct slentry=0.10;
var prin1 prin2 prin3 prin4 prin5;
class present;
run;
proc discrim data=lib.prinjul outstat=dart1 method=normal all;
priors proportional;
class present;
var logvnos;
run;
proc discrim data=lib.prinjul outstat=dart2 method=normal all;
priors proportional;
class present;
var prin2 prin1;
run:
proc discrim data=lib.prinoct outstat=dart3 method=normal all;
priors proportional;
class present;
var logvavg tranvc3 tranvc1 ph;
run;
proc discrim data=lib.prinoct outstat=dart4 method=normal all;
priors proportional;
class present;
var prin1 prin2 prin3;
```

run;

STEPWISE.SAS

```
libname lib 'c:\saswork';
data sortjul1; set lib.prinjul;
no=-1.71631-2.22288*logvnos;
yes=-3.41506-4.34324*logvnos;
logdart=log10(darter+1);
run;
data sortjul2; set sortjul1;
if present=0;
run;
data sortjul4; set sortjul1;
 if present=1;
run;
data sortjul3; set sortjul2;
if yes GE no then fish=1;
else fish=0;
run;
data sortjul5; set sortjul3;
if fish=1;
run;
data sortjul6;
set sortjul5 sortjul4;
run;
proc univariate data=sortjul1 normal plot;
var logdart;
run;
proc stepwise data=sortjul1;
  model logdart=depth ph vegcom1 vegcom2 vegcom3 vbot vavg sc temp do;
run;
proc stepwise data=sortjul6;
 model logdart=depth ph vegcom1 vegcom2 vegcom3 vbot vavg sc temp do;
 run;
 data nozero; set sortjul1;
 if present > 0;
```

```
run;
proc stepwise data=nozero;
model logdart=depth ph vegcom1 vegcom2 vegcom3 vbot vavg sc temp do;
run;
data sortjp1; set lib.prinjul;
no=-1.16546+0.38529*prin2+0.28096*prin1;
ves=-0.56752-0.24080*prin2-0.17560*prin1;
logdart=log10(darter+1);
run;
data sortjp2; set sortjp1;
if present=0;
run;
data sortjp4; set sortjp1;
 if present=1;
run;
data sortjp3; set sortjp2;
if yes GE no then fish=1;
 else fish=0;
run;
data sortjp5; set sortjp3;
if fish=1;
run;
data sortip6;
set sortjp5 sortjp4;
run;
proc stepwise data=sortjp1;
  model logdart=prin1 prin2 prin3 prin4 prin5;
run;
proc stepwise data=sortjp6;
 model logdart=prin1 prin2 prin3 prin4 prin5;
run;
 data jpzero; set sortjp1;
 if present > 0;
 run;
```

```
proc stepwise data=ipzero;
model logdart=prin1 prin2 prin3 prin4 prin5;
run;
data sortoct1; set lib.prinoct;
no=-531.24866+7.33288*logvavg-7.60364*tranvc3-2.78356*tranvc1
+144.95059*ph;
yes=-518.76194+7.35256*logvavg-5.54641*tranvc3+.00135*tranvc1
+143.01975*ph;
logdart=log10(darter+1);
run:
data sortoct2; set sortoct1;
if present=0;
run;
data sortoct4; set sortoct1;
 if present=1;
run;
data sortoct3; set sortoct2;
if yes GE no then fish=1;
else fish=0;
run;
data sortoct5; set sortoct3;
if fish=1;
run;
data sortoct6;
set sortoct5 sortoct4;
run;
proc univariate data=sortoct1 normal plot;
var logdart darter;
run;
proc stepwise data=sortoct1;
 model logdart=depth ph vegcom1 vegcom2 vegcom3 vbot vavg sc temp do;
run;
proc stepwise data=sortoct6;
model logdart=depth ph vegcom1 vegcom2 vegcom3 vbot vavg sc temp do;
run;
```

```
data octzero; set sortoct1;
if present > 0;
run;
proc stepwise data=octzero;
model logdart=depth ph vegcom1 vegcom2 vegcom3 vbot vavg sc temp do;
run;
data sortop1; set lib.prinoct;
no=-0.85760+0.15576*prin1+0.45401*prin2+0.31640*prin3;
yes=-.95972-0.16730*prin1-0.48764*prin2-0.33984*prin3;
logdart=log10(darter+1);
run;
data sortop2; set sortop1;
if present=0;
run;
data sortop4; set sortop1;
 if present=1;
run;
data sortop3; set sortop2;
if yes GE no then fish=1;
else fish=0;
run;
data sortop5; set sortop3;
if fish=1;
run;
data sortop6;
set sortop5 sortop4;
run;
proc stepwise data=sortop1;
 model logdart=prin1 prin2 prin3 prin4 prin5;
run;
proc stepwise data=sortop6;
model logdart=prin1 prin2 prin3 prin4 prin5;
run;
data opzero; set sortop1;
```

if present > 0;
run;

proc stepwise data=opzero; model logdart=prin1 prin2 prin3 prin4 prin5; run;

REGTEST.SAS

```
libname lib 'c:\SASWORK';
data sortiul1; set lib.prinjul;
no=-1.71631-2.22288*logvnos;
yes=-3.41506-4.34324*logvnos;
logdart=log10(darter+1);
run;
proc reg data=sortjul1;
 model logdart=vbot vegcom1 vegcom3 temp;
run;
data julfin; set sortjul1;
predict=2.845638-0.259062*vbot+.008261*vegcom1+.004394*vegcom3-.099353*temp;
run;
proc reg data=julfin;
model logdart=predict / noint;
run;
data aprilfin; set lib.prinapr;
no=-1.71631-2.22288*logvnos;
yes=-3.41506-4.34324*logvnos;
logdart=log10(darter+1);
predict=2.845638-0.259062*vbot+.008261*vegcom1+.004394*vegcom3-.099353*temp;
run;
proc reg data=aprilfin;
 model logdart=predict / noint;
run;
data summer; set julfin aprilfin;
run;
proc glm data=summer;
class period;
model logdart=period predict period*predict;
means period/regwq;
run;
```

```
data sortip1; set lib.prinjul;
no=-1.16546+0.38529*prin2+0.28096*prin1;
yes=-0.56752-0.24080*prin2-0.17560*prin1;
logdart=log10(darter+1);
run;
proc reg data=sortip1;
 model logdart=prin2 prin1 prin5;
run;
data julfinp; set sortjp1;
predict=.533741-.121682*prin3-.093519*prin1-.115021*prin5;
run;
proc reg data=julfinp;
model logdart=predict / noint;
run;
data aprilfp; set lib.prinapr;
no=-1.16546+0.38529*prin2+0.28096*prin1;
yes=-0.56752-0.24080*prin2-0.17560*prin1;
logdart=log10(darter+1);
predict=.533741-.121682*prin3-.093519*prin1-.115021*prin5;
run;
proc reg data=aprilfp;
model logdart=predict / noint;
run;
data summerp; set julfinp aprilfp;
run;
proc glm data=summerp;
class period;
model logdart=period predict period*predict;
means period/regwq;
run;
data sortoct1; set lib.prinoct;
logdart=log10(darter+1);
run;
```

```
proc reg data=sortoct1;
 model logdart=vbot vegcom1 vegcom3;
run;
data octfin; set sortoct1;
predict=.13866-.280402*vbot+.008144*vegcom1+.004486*vegcom3;
run;
proc reg data=octfin;
model logdart=predict / noint;
run;
data janfin; set lib.prinjan;
logdart=log10(darter+1);
predict=.13866-.280402*vbot+.008144*vegcom1+.004486*vegcom3;
run;
proc reg data=janfin;
model logdart=predict / noint;
run;
data fall; set octfin janfin;
run;
proc glm data=fall;
class period;
model logdart=period predict period*predict;
means period/regwq;
run;
data sortop1; set lib.prinoct;
logdart=log10(darter+1);
run;
proc reg data=sortop1;
 model logdart=prin1 prin3 prin4 prin2;
run;
data octfinp; set sortop1;
predict=.277441-.069066*prin1-.072763*prin3+.088007*prin4-.092371*prin2;
run;
```

```
proc reg data=octfinp;
model logdart=predict / noint;
run;
data janfinp; set lib.prinjan;
logdart=log10(darter+1);
predict=.277441-.069066*prin1-.072763*prin3+.088007*prin4-.092371*prin2;
run;
proc reg data=janfinp;
model logdart=predict / noint;
run;
data fallprin; set octfinp janfinp;
run;
proc glm data=fallprin;
class period:
model logdart=period predict period*predict;
means period/regwq;
run;
data allcont; set summer fall;
run;
proc glm data=allcont;
class period;
model logdart=period;
means period/regwq;
run;
proc reg data=sortjul1;
 model logdart=vbot vegcom1 vegcom3 /collin;
 output out=work
 r=resid
 p=predict;
run;
proc univariate data=work normal plot;
var resid;
run;
```

proc plot data=work; plot resid*predict; run;

DFATEST.SAS

```
libname lib 'c:\SASWORK';
data sortjul1; set lib.prinjul;
no=-1.71631-2.22288*logvnos;
yes=-3.41506-4.34324*logvnos;
logdart=log10(darter+1);
run;
data juldfa; set sortjul1;
if no>yes then dfapred=0; else dfapred=1;
run;
proc print data=juldfa;
var strata grid present dfapred;
run;
data aprilfin; set lib.prinapr;
no=-1.71631-2.22288*logvnos;
yes=-3.41506-4.34324*logvnos;
logdart=log10(darter+1);
predict=2.845638-0.259062*vbot+.008261*vegcom1+.004394*vegcom3-.099353*temp;
run;
data aprildfa; set aprilfin;
if no>yes then dfapred=0; else dfapred=1;
run;
proc print data=aprildfa;
var strata grid present dfapred;
run;
data sortjp1; set lib.prinjul;
no=-1.16546+0.38529*prin2+0.28096*prin1;
yes=-0.56752-0.24080*prin2-0.17560*prin1;
logdart=log10(darter+1);
run;
data juldfap; set sortjp1;
if no>yes then dfapred=0; else dfapred=1;
run;
```

```
proc print data=juldfap;
var strata grid present dfapred;
run;
data aprilfp; set lib.prinapr;
no=-1.16546+0.38529*prin2+0.28096*prin1;
yes=-0.56752-0.24080*prin2-0.17560*prin1;
logdart=log10(darter+1);
predict=.533741-.121682*prin3-.093519*prin1-.115021*prin5;
run;
data aprdfap; set aprilfp;
if no>yes then dfapred=0; else dfapred=1;
run;
proc print data=aprdfap;
var strata grid present dfapred;
run;
data sortoct1; set lib.prinoct;
no=-531.24866+7.33288*logvavg-7.60364*tranvc3-2.78356*tranvc1
+144.95059*ph;
yes=-518.76194+7.35256*logvavg-5.54641*tranvc3+.00135*tranvc1
+143.01975*ph;
logdart=log10(darter+1);
run;
data octdfa; set sortoct1;
if no>yes then dfapred=0; else dfapred=1;
run:
proc print data=octdfa;
var strata grid present dfapred;
run;
data janfin; set lib.prinjan;
no=-531.24866+7.33288*logvavg-7.60364*tranvc3-2.78356*tranvc1
+144.95059*ph;
yes=-518.76194+7.35256*logvavg-5.54641*tranvc3+.00135*tranvc1
+143.01975*ph;
logdart=log10(darter+1);
```

```
run;
data jandfa; set janfin;
if no>yes then dfapred=0; else dfapred=1;
run;
proc print data=jandfa;
var strata grid present dfapred;
run;
data sortop1; set lib.prinoct;
no=-0.85760+0.15576*prin1+0.45401*prin2+0.31640*prin3;
yes=-.95972-0.16730*prin1-0.48764*prin2-0.33984*prin3;
logdart=log10(darter+1);
run;
data octdfap; set sortop1;
if no>yes then dfapred=0; else dfapred=1;
run;
proc print data=octdfap;
var strata grid present dfapred;
run;
data janfinp; set lib.prinjan;
no=-0.85760+0.15576*prin1+0.45401*prin2+0.31640*prin3;
yes=-.95972-0.16730*prin1-0.48764*prin2-0.33984*prin3;
logdart=log10(darter+1);
run;
data jandfap; set janfinp;
if no>yes then dfapred=0; else dfapred=1;
run;
proc print data=jandfap;
var strata grid present dfapred;
run;
```